Low plasma taurine levels in English Cocker Spaniels diagnosed with Dilated Cardiomyopathy

Journal:	*Journal of Small Animal Practice*
Manuscript ID	JSAP-2020-0192.R3
Manuscript Type:	Original Paper
Keywords:	Cardiac, Dogs, Amino acid, Deficiency, Supplementation, Concentration

Low plasma taurine levels in English Cocker Spaniels diagnosed with Dilated Cardiomyopathy

Abstract (233 words)

Objective: Dilated cardiomyopathy (DCM) has been documented in Cocker Spaniels. American Cocker Spaniels (ACS) with taurine deficiency and DCM phenotype improved following taurine supplementation. No studies have been performed investigating taurine deficiency in English Cocker Spaniels (ECS). The aims of this study were to evaluate taurine levels in ECS with DCM and assess their survival time and natural progression of their disease.

Methods: Retrospective comparison of ECS with DCM phenotype with and without taurine deficiency at the cardiology department of a UK academic referral centre between 2008 and 2018.

Results: Taurine plasma concentration was available in 16 ECS with DCM phenotype; 13/16 of which had congestive heart failure and 3/16 of which did not. Taurine concentration was low (<50 µmol/L) in 13/16 and normal in 3/16. Deficient dogs received taurine supplementation in addition to conventional cardiac medications. Eight dogs were still alive at the end of this study and 8 were dead. MST for all dogs included in the study was 2800 days. Left ventricular (LV) systolic function improved and LV dimensions reduced in ECS with taurine deficiency following taurine supplementation and conventional cardiac therapy, although similar results were observed in ECS with normal taurine concentration on cardiac therapy alone.

25 *Clinical importance:* Based on laboratory reference intervals, low taurine
26 concentrations were common in ECS with DCM, showing a possible association
27 between DCM in ECS and taurine deficiency; supplementation with taurine was not
28 curative.
29

WORD COUNT: 4157

Introduction

Dilated cardiomyopathy (DCM) is the most common acquired myocardial disease in dogs (Fox *et al.* 1999). Echocardiography is the gold standard for diagnosis: decreased systolic function leads to renin-aldosterone-angiotensin system (RAAS) activation and ventricular dilation which may eventually result in congestive heart failure. Left atrial enlargement and arrhythmias may also be present (Dukes-McEwan *et al.* 2003). The preclinical or occult phase of the disease is characterized by chamber dilation with reduced systolic function and possible arrhythmias with no clinical signs (Dukes-McEwan *et al.* 2003). Medical treatment varies depending on the phase. Pimobendan is recommended for occult DCM (Summerfield *et al.* 2012), but once clinical signs of CHF develop, addition of diuretics and potentially ACE inhibitors and spironolactone, is indicated (Dukes-McEwan 2000; Luis Fuentes *et al.* 2002). Any haemodynamically significant arrhythmias may also require treatment.

Primary (idiopathic) DCM has been documented in a number of breeds including English cocker spaniel (ECS) (Gooding *et al.* 1982, 1986; Thomas 1987; Tidholm *et al.* 1997). In a large UK survey of dogs presenting with DCM, ECS was the fourth most common breed affected, with 30/369 cases and was reported to have longer survival times compared with other breeds (Martin *et al.* 2009).

The DCM phenotype may be a consequence of heritable genetic mutations, viral infections, immune-mediated disorders, arrhythmias, toxins and nutritional deficiencies (Van Vleet and Ferrans, 1986; Cunningham *et al.,* 1992; Shinbane *et al.,*

1997; Backus *et al.* 2006). Due to familial disease, a genetic basis is suspected in some breeds and already documented in others, ~~including ECS (Thomas 1987),~~ as recently reviewed by Dutton & Lopez-Alvarez (2018). Prior to making the diagnosis of DCM, other conditions which may result in similar echocardiographic changes must be actively excluded.

Taurine deficiency has been implicated as a nutritional cause of a DCM phenotype. This was initially reported in a group of cats affected by DCM, where the phenotype completely reversed with taurine supplementation; prior to this discovery, the prognosis for cats with DCM caused by taurine deficiency was grave (Pion *et al.* 1987). Later, American Cocker Spaniels (ACS) with low taurine concentrations were also reported to at least partially reverse their DCM phenotype after both taurine and L-carnitine supplementation (Kittleson *et al.* 1997). Other studies reported similar findings, in this and other breeds such as Golden Retrievers (Kramer *et al.,* 1995; Gavaghan & Kittleson 1997; Kaplan *et al.,* 2018; Ontiveros *et al.,* 2020).

English cocker spaniels were imported into the United States in the 19th century: ACS were developed and eventually recognized as a different breed in 1936 (Fogle 1996). Therefore, there is likely to be a genetic relationship between the ACS studied by Kittleson and colleagues (1997) and the ECS population. To the authors' knowledge, no studies have been reported investigating taurine deficiency in ECS with DCM.

The main aim of this study was to investigate a possible association between taurine deficiency and DCM in ECS in the UK. The hypothesis was that ECS with a

diagnosis of DCM could also have low taurine levels, similar to ACS. Additional aims of this study were to investigate the response to taurine supplementation in deficient ECS and document the progression of DCM and survival times in this breed.

Materials and methods

This was an observational, retrospective study. Cases were retrieved from a single multidisciplinary referral hospital in the UK.

The hospital database was searched for ECS examined by the cardiology service between 2008 and 2018 and diagnosed with DCM. Dogs were included if retrieved data included both a complete echocardiographic examination and plasma taurine concentration. All dogs had indirect assessment of systolic blood pressure (Doppler method). Routine blood work (haematology, biochemistry, thyroid function assessment) was carried out if the clinician considered it relevant to the investigations for each patient.

Dogs with other concurrent cardiac conditions were excluded. Dogs with clinical signs, blood pressure or clinical pathology results indicating significant systemic disease, including systemic hypertension, were excluded. Systemic hypertension was defined as >160mmHg on repeated measurements on more than one occasion, in accordance with the ACVIM guidelines (Acierno *et al.* 2018).

Dogs affected by hypothyroidism, on treatment with levothyroxine, were included provided that the dog had been receiving treatment for over 2 months prior to inclusion and the condition was considered stable on medical therapy, similar to the criteria described by Summerfield *et al.* (2012).

From the patient records, the following data were retrieved: weight, age, gender, neuter status and echocardiography results. Electrocardiograms and results were reviewed, if available. Laboratory data (biochemistry and haematology) were reviewed, where available, to exclude concurrent conditions. Medications and doses prescribed for each patient were also retrieved.

For taurine analysis, heparinised plasma samples were submitted to IDEXX (Referral assay via IDEXX Laboratories, Wetherby, United Kingdom). Samples were centrifuged and plasma separated within 30 minutes of the blood sample being taken. Taurine deficiency was defined as concentrations <50 µmol/L, based on the laboratory's reference range interval (50-180 µmol/L); these were extrapolated from the MUST study (Kittleson *et al.,* 1997) and were also confirmed in others studies that included various breeds (Kramer *et al.,* 1995; Delaney *et al.,* 2003; Tôrres *et al.,* 2003).

Doppler Echocardiographic examinations were carried out using a GE Vivid 7 (Buckinghamshire, UK) machine, using a 7S or M4S transducer. The dogs were in lateral recumbency on a purpose-designed table to allow imaging via the dependent thoracic wall. Studies have been performed by either a cardiology diplomate or a cardiology resident under the direct supervision of a diplomate. Two dimensional (2D) and M-mode images were acquired, recorded and measured according to standard protocols (Sahn *et al.* 1978; Thomas *et al.* 1994; Boon 1998). Data from the M-mode studies retrieved included left ventricular internal dimensions both in diastole (LVIDd) and systole (LVIDs); fractional shortening (FS) was calculated. The M-mode LV diameters were normalised for body weight by allometric scaling in diastole (LVIDDN) and systole (LVIDSN) (Cornell *et al.* 2004). The mitral E point to

septal separation (EPSS) measurement from mitral valve M-mode was also recorded. From the 2D right parasternal long axis 4 chamber view optimizing the left ventricular length and area, Simpson's method of discs was used to determine LV end-diastolic and end-systolic volumes. Ejection fraction (EF), and sphericity index were calculated (Dukes-McEwan *et al.* 2003). The end-systolic and end-diastolic volumes indexed to body surface area (BSA) were also calculated (LVESVi and LVEDVi respectively). The BSA was calculated using the standard formula (Ford & Mazzaferro 2011). Maximal left atrial diameter, measured at the end of ventricular systole from a right parasternal long-axis 4 chamber view and the short axis ratio of the left atrium to aortic diameters, measured at the end of diastole, were recorded (Chetboul & Tissier 2012). Colour flow and spectral Doppler were used to exclude other significant cardiac diseases. Mitral regurgitation was accepted provided it was a central jet implying origin due predominantly to stretch of the mitral annulus, rather than primary mitral valve disease (myxomatous or dysplastic); dogs with markedly thickened or prolapsing mitral leaflets were not included. Colour flow and spectral Doppler transvalvular flows were documented, but not analysed further for purposes of this study.

Repeated echocardiographic studies were obtained at a frequency determined by the attending clinician, and the echocardiographic data were retrieved from every available examination.

Congestive heart failure was defined as left-sided if there were compatible radiographic findings, when available; in the absence of radiographs, echocardiographic signs of increased left filling pressures (Schober *et al.,* 2010) in association with clinical signs and response to furosemide administration were

considered supportive of CHF. Radiographs had been reviewed and reported by diagnostic imaging diplomates or diagnostic imaging residents working under supervision of a diplomate.

If dogs had plasma taurine level <50 µmol/L, supplementation with taurine was commenced. Dogs with CHF or with preclinical DCM were treated according to the individual clinician and owner preference. Drugs used and their doses were recorded.

Survival time was calculated from the time of initial diagnosis of DCM and taurine assay to death. Cardiac deaths were defined as sudden death or euthanasia because of cardiac reasons. Other causes of death were categorised as non-cardiac. Dogs lost to follow-up were censored.

Statistical analysis

All analyses were performed with Graphpad Prism 7 (GraphPad Software, Inc, La Jolla, California, US). Data were inspected graphically for normality of distribution and tested for normality with a Shapiro-Wilk test. Continuous data are presented as mean ± standard deviation when normally distributed, or as median and interquartile range (IQR; 25^{th} – 75^{th} percentile) when not normally distributed.

Survival time was evaluated for dogs with low and normal taurine levels. A Kaplan-Meier curve was constructed. Dogs were right censored if still alive, lost to follow-up or if they had died of non-cardiac disease.

Results

Sixty ECS were evaluated by the cardiology referral service of an academic institution between 2008 and 2018.

Forty-four dogs were excluded from the study. Thirty-three of these were diagnosed with other cardiac diseases. Eleven dogs were excluded due to insufficient data; of these, 3 dogs were reported to have DCM but no information regarding taurine levels was available.

Sixteen dogs met the inclusion criteria: 13/16 had low plasma taurine concentration. In the dogs with low plasma taurine, the mean taurine concentration was 17.46 ± 11.03 µmol/L. Three dogs had normal taurine concentrations (75, 81 and 194 µmol/L). Thirteen dogs were in congestive heart failure. The 3/16 dogs which did not have CHF all had low taurine concentrations.

The mean age of the dogs included in the study was 6.75 ± 3.02 years, the mean body weight was 15.3 kg ± 2.7. There were 11 males and 5 females included. There were 8 males (4 neutered) and 5 females (4 neutered) with low taurine levels. All dogs with normal taurine levels were males (2 neutered). Signalment, taurine concentrations, CHF status and medications including taurine supplementation and outcome at the time of writing are reported for each individual dog in Table 1.

Taurine supplementation was started in all dogs with low taurine concentration at a dose of 67.8 ± 38.9 mg/kg/day. Eleven dogs did not have taurine levels rechecked, though the 2 dogs with low taurine levels that did have further measurements 6

months later showed values of 200 and 279 µmol/L (ref. 50-180). All dogs received one or more cardiac medications; 8 dogs were receiving other medications or supplements (Table 1).

Two dogs with low taurine concentrations and one with normal taurine levels received clopidogrel due to left atrial spontaneous echocontrast, suspected to represent a hypercoagulable state. The dog with normal taurine also received doxycycline due to the presence of ticks and the fact that tick-borne disease could not be ruled-out. The same dog received sildenafil to treat pulmonary hypertension presumed secondary to left-sided CHF. Another dog with low taurine levels received amlodipine in the attempt of afterload reduction.

Echocardiographic variables at admission and at follow-up (median 30 days; range 7-90) were reported (Table 2). Serial echocardiographic studies were available for 10/16 dogs. Comparison between echocardiographic variables at baseline and at the first follow-up are shown (Fig. 1). Figure 2 shows echocardiographic images of one of the dogs with low taurine concentration with dilated left ventricle and poor systolic function before (Fig. 2a-2b) and after (Fig. 2c-2d) taurine supplementation; is improvement of left ventricular dimensions and systolic function at the recheck although statistical comparison was not performed ~~(Before: EF: 38%; FS: 11%; LVESVi: 102.9 mL/m^2; LVIDSN: 2.07; LVIDDN: 2.47. After: EF: 47%; FS: 7%; LVESVi: 64.2 mL/m^2; LVIDSN: 1.07; LVIDDN: 1.93)~~. In all dogs included in the study, there was a subjective improvement between admission and first re-check values of LVESVi, LVIDSN, LVIDDN and EF. The dogs with low taurine levels showed a subjective improvement between admission and re-check values of LVIDSN and LVIDDN, but not in LVESVi and EF. Again, all the above values were

not statistically compared due to low numbers and to avoid “testing against baseline”. For the dogs which underwent serial echocardiographic examinations, graphical representation of LVESVi and LVIDSN values over time are shown in figures 3a and 3b, respectively (Fig. 3a-b).

All dogs that died before the end of the study were euthanized due to worsening of their cardiac disease (Table 1). Four dogs were lost to follow-up (all had low taurine concentrations, 3 were in CHF).

The median survival time (MST) for all dogs included in the study was 1155 days (195 -2800) (Fig. 4). Dogs with low taurine levels had a MST of 2800 days (790 – upper limit not calculable), whereas those with normal levels had a survival time of 14, 90 and 478 days. The 13 dogs ~~in~~ with CHF (10 with low taurine levels and 3 with normal levels) had a MST of 1155 days (478-2800), whereas the two non-CHF dogs survived for 83 and 840 days, respectively (one dog was lost to follow-up).

Discussion

Based on our laboratory reference range, we found that taurine deficiency is commonly identified in ECS diagnosed with DCM. However, no clear causal association could be identified in this study; indeed, the study design does not allow causal relationships to be investigated.

~~The serial echocardiographic data shows that taurine supplementation might not be curative and taurine deficiency may not be the sole cause of DCM phenotype in this breed.~~ In dogs with serial echocardiographic data, we did not carry out any statistical analysis in view of small numbers in this descriptive study. However, data suggest

that taurine supplementation might not be curative and taurine deficiency may not be the sole cause of DCM phenotype in this breed. This has also been shown in other breeds such as ACS, Golden retrievers, Newfoundlands and Irish Wolfhounds (Kittleson *et al.* 1997; Fascetti *et al.* 2003; Alroy *et al.* 2005; Bélanger *et al.* 2005; Backus *et al.* 2006; Vollmar *et al.* 2013). In contrast, cats with taurine-deficient~~cy~~ DCM have a reversible cardiomyopathy with taurine supplementation (Pion et al. 1987).

We did not measure the whole blood taurine concentrations and these have been reported to be substantially higher than plasma taurine concentrations ~~(Delaney et al., 2003).~~ Whole blood taurine concentration may be superior, if available, as it more closely reflects muscle taurine concentration and therefore overall taurine status, whereas plasma taurine may reflect fasting or post-prandial status (Delaney et al 2003). For this study, only plasma taurine concentrations could be assayed and no record were made of when each dog's last meal had been taken prior to sampling.

As mentioned above, no statistical analysis was performed between admission and re-check echocardiography values in order to avoid "testing against baseline", therefore only subjective or visual assessments could be made; however, it is interesting to notice changes that we recorded in our dogs during the study period. As figures 1 a-h and 3 a-b show, at the first follow-up echocardiography values showed reduction in LV diameter and volumes (LVIDd, LVESVi, LVIDDN, LVIDSN) with improved systolic function (EF, LVESVi) if the whole population was considered. However, those with low taurine levels at the re-check, had an improvement in LVIDd, LVIDDN and LVIDSN but not in LVESVi and EF. In line with our data, Kittleson and colleagues (1997) reported that ACS with DCM and low taurine

concentrations showed improved systolic function after supplementation. Taurine supplementation may improve systolic function, even in the absence of a taurine deficient state as shown in. In a study conducted in people with chronic CHF, where taurine supplementation was given for 6 weeks and a substantial improvement in systolic function was reported (Azuma *et al.* 1992). Therefore, it is possible that taurine supplementation at pharmacological doses, could have played a role in the reduction of the LV chamber dimensions and improvement in systolic function in our population of ECS, even if low-taurine status was not associated with their DCM. Since all ECSs also received conventional cardiac therapy, it is not possible to separate the effects of this medications from taurine supplementation in ECS with low taurine concentrations. Diuretics reduce preload, which will reduce LV size (showed by a reduction in values of LVIDd), as well as resolving fluid retention associated with CHF due to both systolic dysfunction and RAAS activation. It is also well documented that pimobendan reduces ventricular size in both CHF and preclinical DCM patients as well as dogs with mitral valve myxomatous disease (Summerfield *et al.* 2012; Häggström *et al.* 2013; Boswood *et al.* 2016).

A relationship between taurine deficiency and DCM phenotype in ACS was initially reported by Kramer et al. (1995). A few years later, in the multicentred spaniel trial (MUST) study, Kittleson and colleagues (1997) showed an improved systolic function in the breed following supplementation with both taurine and L-carnitine. Unfortunately, the concurrent use of both supplements makes it unclear whether the response observed was due to the concurrent L-carnitine supplementation. In our study, myocardial L-carnitine levels were not assessed, as myocardial biopsies are required for diagnosing carnitine deficiency (Meurs, 2004) and L-carnitine was only

supplemented in one dog (dog 4 in Table 1), who died a cardiac death 115 days after diagnosis without a follow-up echocardiography. It is therefore possible that different results may had been achieved if L-carnitine was also routinely supplemented to the low taurine dogs, which would then also allow a direct comparison with the MUST study (Kittleson et al 1997). Indeed, one of the reasons why L-carnitine was started in the MUST study population was because the first 2 ACS failed to reach demonstrable improvement with taurine alone, despite normal plasma levels of L-carnitine (Kittleson et al 1997). Taurine synthesis has also been shown to differ between breeds, with large breeds more predisposed to deficiency: groups of Newfoundlands and Golden Retrievers have been reported to have low taurine concentration and a DCM phenotype, which improved after taurine supplementation (Fascetti *et al.* 2003; Bélanger *et al.*2005*;* Backus *et al.* 2006).

In cats, taurine deficiency can be associated with several potential causes including increased excretion with urine and faeces (Hickman *et al.,* 1992; Edgar *et al.* 1998). In our study, urinary and faecal taurine concentration were not measured. Another explanation for taurine deficiency is related to diet. ~~C~~ and consumption of certain commercial and prescription diets have been implicated with low plasma taurine concentrations in dogs with DCM (Sanderson *et al.* 2001; Fascetti *et al.* 2003; Sanderson, 2006; Ko *et al.* 2007; Kaplan *et al.* 2018).

More recently, grain free diets and exotic ingredients have been suspected to be associated with DCM phenotype (Freeman *et al.* 2018) although not always with low taurine concentrations. Unfortunately, we were unable to retrieve diet history for all the ECS due to the retrospective nature and long time-course of our study.

Two dogs with low taurine levels and one with normal taurine levels received clopidogrel due to the presence of left atrial spontaneous echocontrast. This can also be associated with low velocity blood flow or inflammatory disease and both conditions can lead to thrombus formation. (Spence *et al.,* 2019)

In this study, ECS affected by DCM and CHF had a MST of 1155 days~~. This~~ which is ~~much~~ longer than the survival time associated with DCM and CHF reported in ~~the literature~~other breeds. A survival time of 27 days was reported in 189 dogs of various breeds with DCM and CHF whereas a MST of 65 days was found in a group of 37 dogs affected by DCM; in both these studies, dogs did not receive pimobendan (Monnet *et al.* 1995; Tidholm *et al.* 1997).

More recent data showed a MST of 133 days in 369 dogs of various breeds with DCM (74% in CHF at presentation) (Martin *et al.,*2009). Dobermanns in CHF were also shown to have a short MST of 50.67 days that increased to 329 days with pimobendan therapy (Luis Fuentes *et al.,* 2002). Dobermanns with preclinical DCM at presentation had times to primary end-point (sudden death or CHF) of 441 days which was shown to increase to 718 days in dogs receiving pimobendan (Summerfield *et al.* 2012). American Cocker Spaniels with DCM and low-taurine in the MUST study (Kittleson *et al.* 1997) had a longer MST (849 days) than that reported in previous studies, but still shorter than our ECS. Data from an unpublished study state a MST of ECS with DCM of 750 days (P. Wotton, 1998)[1]; however, taurine levels were not measured in these dogs, nor was it supplemented. In the study by Luis Fuentes (2002), ECS receiving placebo or pimobendan had a

[1] Wotton, P.R., (1998). Cardiomyopathy in English cocker and springer spaniels: A review of 38 cases. Proceedings of the British Small Animal Veterinary Association, p.316.

MST of 537 and 1037 days, respectively, showing considerably longer survival time compared to other breeds, which is supported by the results presented here. The most recent comparison of different breeds with DCM showed ECS with DCM to have a MST of 511 days, the longest amongst all the breeds in the study (Pedro *et al.* 2011). It can be appreciated from these studies that the MST may be longer in ECS, compared with other breeds with DCM.

The MST of the dogs with low taurine levels was 2800 days, numerically longer than that reported for ECS with DCM in other publications (511 days, Pedro et al 2011; 750 days, Wotton 1998, unpublished data). Dogs from Dr Wotton's historical study did not receive pimobendan, which might explain the shorter MST. Dobermanns with DCM receiving pimobendan showed a longer MST than those on placebo, but the same study did not show a statistically significant improvement in ECS receiving pimobendan. ~~, perhaps~~ This may be because they survived for longer regardless of treatment, provided the CHF was controlled (Luis Fuentes *et al.* 2002). ~~Nevertheless,~~ ~~o~~Our results may suggest a response to taurine supplementation, however this was only a subjective improvement in a small population in which it was not appropriate to make statistical comparison.

It is possible that once CHF is well managed, ECS may have a more favourable prognosis despite the diagnosis of DCM~~.~~, although ~~T~~ the low numbers of dogs in the pre-clinical phase may have affected these results.

Statistical comparison of MST of dogs in CHF and not in CHF, dogs with low taurine levels and normal taurine levels was not performed due to low numbers that would have led to unreliable results.

Limitations

This study has some limitations due to its retrospective nature. Firstly, we had a small number of cases and this could have affected the reliability of the results. The low numbers of ECS with DCM with normal taurine concentration mean it was not possible to compare aspects about DCM or response to treatment in these dogs and the dogs with low taurine concentrations.

We did not compare echocardiographic values between baseline and recheck to avoid "testing against baseline", therefore, the above results should be considered as subjective based on visual assessments of the graphs. The echocardiographic examinations were performed by different operators and inter-operator and inter-observer variability were not assessed as part of this study. However, all echocardiographers had undergone similar training and followed similar acquisition and measurement protocols.

Histopathology was not performed in any of the cases included in the study, therefore, the diagnosis was based on echocardiographic findings. We also did not obtain pedigree information from these dogs, so we were not able to investigate for familial DCM, or possible inherited basis for the taurine deficiency. This should be addressed in future prospective studies.

Dogs were classified as taurine deficient based on the laboratory reference interval~~.~~ but ~~B~~breed specific reference range is currently not available. Ideally, taurine concentration should have been tested in a control group of ECS without DCM since it is possible that this breed has different basal plasma taurine levels as

demonstrated in Golden Retrievers (Ontiveros *et al.,* 2020). Also, whole blood taurine concentrations were not measured in this study.

We did not investigate the type of diet the dogs were fed so we cannot assess the association between diet and DCM in this study (Freeman *et al.,* 2018).

Taurine plasma concentrations after supplementation were not measured in most dogs, therefore the effectiveness of supplementation cannot be confirmed. However, in those with taurine concentrations rechecked after supplementation, increased taurine values were recorded. Moreover, in the MUST study (Kittleson et al. 1997), all ACS had increased taurine concentrations with similar dose of supplementation as in our study. Furthermore, a study in Newfoundlands showed that taurine supplementation at any dose normalised blood taurine levels and higher doses were associated with increased urinary taurine loss and no changes in plasma or whole blood taurine concentrations (Dukes-McEwan et al., 2001).

~~Moreover~~An additional limitation was that plasma or myocardial carnitine concentrations were not measured and supplementation was started in only one dog making direct comparison with the MUST study impossible.

We were unable to determine if the improvements in echocardiographic variables were secondary to the taurine supplementation or due to the other standard cardiac medications; treating dogs with only taurine supplementation would be ethically unacceptable. Moreover, the treatment of dogs was not standardized, although most of the patients were receiving similar medications for CHF. The dogs with normal taurine concentrations (3) were in CHF and this could affect the survival analysis leading to a longer MST for dogs with low taurine concentration (10/13 in CHF). We did not have a MST value for the non-CHF dogs due to the high number of censored cases (1 out of 3).

Two dogs were receiving diltiazem to treat supraventricular tachycardias. This could have affected the survival analysis. Tachycardiomyopathy was possible though considered less likely since the arrhythmia was diagnosed after the diagnosis of dilated cardiomyopathy; therefore was believed to be secondary to atrial stretch."

Lastly, one dog had treated hypothyroidism, with historical low serum total thyroxine (T4) concentrations. This dog was not excluded from the study since this condition was considered stable and the dog had been treated with levothyroxine for 4 months prior to inclusion.

Conclusions

In conclusion, this study has revealed that taurine deficiency is common in ECS affected by DCM; taurine status should be checked in this breed if a diagnosis of DCM is made. Based on the current study, a direct association between these two conditions could not be established but it is suspected. We provided further evidence that ECS have a longer survival time than other breeds with DCM, especially those with taurine deficiency who are supplemented.

A larger prospective study is needed to confirm the incidence of taurine deficiency in ECS and its association with DCM. The role of supplementing L-carnitine concurrently should also be explored. In particular, including a detailed diet history in prospective assessments will be essential.

449 No conflicts of interest have been declared
450

REFERENCES

Acierno, M.J., Brown, S., Coleman, A.E., *et al.* (2018) ACVIM consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *Journal of veterinary internal medicine* **32**, 1803–1822

Alroy, J., Rush, J.E. & Sarkar, S. (2005) Infantile dilated cardiomyopathy in Portuguese water dogs: correlation of the autosomal recessive trait with low plasma taurine at infancy. *Amino acids* **28**, 51–56

Azuma, J., Sawamura, A. & Awata, N. (1992) Usefulness of Taurine in Chronic Congestive Heart Failure and Its Prospective Application : current therapy of intractable congestive heart failure. *Japanese circulation journal* **56**, 95–99

Backus, R.C., Ko, K.S., Fascetti, A.J., *et al.,* (2006) Low plasma taurine concentration in Newfoundland dogs is associated with low plasma methionine and cysteine concentrations and low taurine synthesis. *The Journal of nutrition*, 136(10), 2525-2533.

Bélanger, M.C., Ouellet, M., Queney, G., *et al.* (2005) Taurine-deficient dilated cardiomyopathy in a family of golden retrievers. *Journal of the American Animal Hospital Association* **41**, 284–291

Boon, J.A. (1998) The M-Mode and Doppler Examination. In: *Manual of veterinary echocardiography*. 2nd edn. Eds Williams & Wilkins. pp 139-205

Boswood, A., Häggström, J., Gordon, S.G., *et al.* (2016) Effect of Pimobendan in Dogs with Preclinical Myxomatous Mitral Valve Disease and Cardiomegaly: The EPIC Study-A Randomized Clinical Trial. *Journal of Veterinary Internal Medicine, 30(6), pp.1765-1779.*

Calvert, C.A., Pickus, C.W., Jacobs, G.J., *et al.* (1997) Signalment, survival, and prognostic factors in Doberman Pinschers with end-stage cardiomyopathy. *Journal of veterinary internal medicine* **11**, 323–326

Chetboul, V. & Tissier, R. (2012) Echocardiographic assessment of canine degenerative mitral valve disease. *Journal of veterinary cardiology: the official journal of the European Society of Veterinary Cardiology* **14**, 127–148

Cornell, C.C., Kittleson, M.D., Della Torre, P., *et al.* (2004) Allometric scaling of M-mode cardiac measurements in normal adult dogs. *Journal of veterinary internal medicine* **18**, 311–321

Dukes-McEwan, J.D. (2000) Canine dilated cardiomyopathy 2. Pathophysiology and treatment. *In Practice, 22(10), pp.620-628.*

Dukes McEwan, J., Biourge, V., Ridyard, A., *et al.* (2001). Dilated cardiomyopathy (DCM) in Newfoundland dogs: Association with low whole blood taurine level. BSAVA Congress 2001 Scientific Proceedings Abstract 14. p. 500. Also: *Journal of Small Animal Practice* **42**; 365.

Dukes-McEwan, J., Borgarelli, M., Tidholm, A., *et al.* (2003) Proposed guidelines for the diagnosis of canine idiopathic dilated cardiomyopathy. *Journal of veterinary cardiology,* **5**, 7–19

Dutton, E. & López-Alvarez, J. (2018) An update on canine cardiomyopathies - is it all in the genes? *The Journal of small animal practice, 59(8), pp.455-464.*

Edgar, S.E., Kirk, C.A., Rogers, Q.R., *et al.* (1998) Taurine status in cats is not maintained by dietary cysteinesulfinic acid. *The Journal of nutrition* **28**, 751–757

Fascetti, A.J., Reed, J.R., Rogers, Q.R., *et al.* (2003) Taurine deficiency in dogs with dilated cardiomyopathy: 12 cases (1997–2001). *Journal of the American Veterinary Medical Association* **223**, 1137–1141

Fogle, B. (1996) Cocker Spaniel, American & English. Willowdale: Firefly Books.

Ford, R.B. & Mazzaferro, E. (2011) Charts and tables. In: *Kirk & Bistner's Handbook of Veterinary Procedures and Emergency Treatment* - 9th edn. Elsevier Health Sciences. pp 646

Fox, P.R., Sisson, D. & Sydney Moïse, N. (1999) Myocardial diseases of dogs. In: Textbook of Canine and Feline Cardiology: Principles and Clinical Practice. 2nd edn. Eds W B Saunders Company. pp 581-619

Freeman, L.M., Stern, J.A., Fries, R., *et al.* (2018) Diet-associated dilated cardiomyopathy in dogs: what do we know? *Journal of the American Veterinary Medical Association* **253**, 1390–1394

Gavaghan, B.J. & Kittleson, M.D. (1997) Dilated cardiomyopathy in an American Cocker Spaniel with taurine deficiency. *Australian Veterinary Journal, 75*(12), pp.862-868

Gooding, J.P., Robinson, W.F. & Mews, G.C. (1986) Echocardiographic characterization of dilatation cardiomyopathy in the English cocker spaniel. *American journal of veterinary research* **47**, 1978–1983

Gooding, J.P., Robinson, W.F., Wyburn, R.S., *et al.* (1982) A cardiomyopathy in the English Cocker Spaniel: a clinico-pathological investigation. *Journal of Small Animal Practice, 23(3), pp.133-149.*

Häggström, J., Boswood, A., O'Grady, M., *et al.* (2013) Longitudinal analysis of quality of life, clinical, radiographic, echocardiographic, and laboratory variables in dogs with myxomatous mitral valve disease receiving pimobendan or benazepril: the QUEST study. *Journal of veterinary internal medicine* **27**, 1441–1451

Hickman, M.A., Bruss, M.L., Morris, J.G., *et al.* (1992) Dietary protein source (soybean vs. casein) and taurine status affect kinetics of the enterohepatic circulation of taurocholic acid in cats. *The Journal of nutrition* **122**, 1019–1028

Kaplan, J.L., Stern, J.A., Fascetti, A.J., *et al.* (2018) Taurine deficiency and dilated cardiomyopathy in golden retrievers fed commercial diets. *PloS one* 13(12), p.e0209112.

Kittleson, M.D., Keene, B., Pion, P.D., *et al.* (1997) Results of the Multicenter Spaniel Trial (MUST): Taurine-and Carnitine-Responsive Dilated Cardiomyopathy in American Cocker Spaniels With Decreased Plasma Taurine Concentration. *Journal of Veterinary Internal Medicine, 11(4), pp.204-211.*

Ko, K.S., Backus, R.C., Berg, J.R., *et al.* (2007) Differences in taurine synthesis rate among dogs relate to differences in their maintenance energy requirement. *The Journal of nutrition* **137**, 1171–1175

Kramer, G.A., Kittleson, M.D., Fox, P.R., *et al.* (1995) Plasma Taurine Concentrations in Normal Dogs and in Dogs With Heart Disease. *Journal of Veterinary Internal Medicine, 9(4), pp.253-258.*

Luis Fuentes, V., Corcoran, B., French, A.*et al.* (2002) A double-blind, randomized, placebo-controlled study of pimobendan in dogs with dilated cardiomyopathy. *Journal of veterinary internal medicine,* 16(3), 255-261.

Martin, M.W.S., Stafford Johnson, M.J. & Celona, B. (2009) Canine dilated cardiomyopathy: a retrospective study of signalment, presentation and clinical findings in 369 cases. *The Journal of small animal practice* **50**, 23–29

Meurs, K.M., Miller, M.W. & Wright, N.A. (2001) Clinical features of dilated

549 cardiomyopathy in Great Danes and results of a pedigree analysis: 17 cases
550 (1990-2000). *Journal of the American Veterinary Medical Association* **218**, 729–
551 732
552 Meurs, K.M., (2004). Boxer dog cardiomyopathy: an update. *Veterinary Clinics:*
553 *Small Animal Practice*, 34(5), pp.1235-1244.
554 Monnet, E., Orton, E.C., Salman, M., *et al.* (1995) Idiopathic dilated cardiomyopathy
555 in dogs: survival and prognostic indicators. *Journal of veterinary internal*
556 *medicine* **9**, 12–17
557 Ontiveros, E. S., Whelchel, B. D., Yu, J., *et al.* (2020). Development of plasma and
558 whole blood taurine reference ranges and identification of dietary features
559 associated with taurine deficiency and dilated cardiomyopathy in golden
560 retrievers: A prospective, observational study. *Plos one,* 15-5
561 Pedro, B.M., Alves, J.V., Cripps, P.J., *et al.* (2011) Association of QRS duration and
562 survival in dogs with dilated cardiomyopathy: a retrospective study of 266 clinical
563 cases. *Journal of veterinary cardiology,* **13**, 243–249
564 Pion, P.D., Kittleson, M.D., Rogers, Q.R., *et al.* (1987) Myocardial failure in cats
565 associated with low plasma taurine: a reversible cardiomyopathy. *Science* **237**,
566 764–768
567 Rishniw, M., & Erb, H. N. (2000). Evaluation of four 2-dimensional echocardiographic
568 methods of assessing left atrial size in dogs. *Journal of Veterinary Internal*
569 *Medicine*, 14(4), 429-435.
570 Sahn, D.J., DeMaria, A., Kisslo, J., *et al.* (1978) Recommendations regarding
571 quantitation in M-mode echocardiography: results of a survey of
572 echocardiographic measurements. *Circulation* **58**, 1072–1083
573 Sanderson, S.L., Osborne, C.A., Lulich, J.P., *et al.* (2001) Evaluation of urinary
574 carnitine and taurine excretion in 5 cystinuric dogs with carnitine and taurine
575 deficiency. *Journal of veterinary internal medicine* **15**, 94–100
576 Sanderson, S.L., (2006) Taurine and carnitine in canine cardiomyopathy. *Veterinary*
577 *Clinics: Small Animal Practice*, 36(6), pp.1325-1343.
578 Schober, K.E., Hart, T.M., Stern, *et al.* (2010) Detection of congestive heart failure in
579 dogs by Doppler echocardiography. *Journal of Veterinary Internal Medicine,*
580 24(6), 1358-1368.
581 Shinbane, J.S., Wood, M.A., Jensen *et al.* (1997). Tachycardia-induced
582 cardiomyopathy: a review of animal models and clinical studies. *Journal of the*
583 *American College of Cardiology*, 29(4), 709-715.
584 Spence, S., French, A., Penderis, J. *et al.* (2019). The occurrence of cardiac
585 abnormalities in canine steroid-responsive meningitis arteritis. *Journal of Small*
586 *Animal Practice,* 60, 204-211.
587 Strohm, L.E., Visser, L.C., Chapel, E.H.,*et al.* (2018) Two-dimensional, long-axis
588 echocardiographic ratios for assessment of left atrial and ventricular size in
589 dogs. *Journal of Veterinary Cardiology*, 20, 330-342.
590 Smith, D.N., Bonagura, J.D., Culwell, N.M., *et al.* (2012) Left ventricular function
591 quantified by myocardial strain imaging in small-breed dogs with chronic mitral
592 regurgitation. *Journal of veterinary cardiology: the official journal of the European*
593 *Society of Veterinary Cardiology* **14**, 231–242
594 Summerfield, N.J., Boswood, A., O'Grady, M.R., *et al.* (2012) Efficacy of
595 pimobendan in the prevention of congestive heart failure or sudden death in
596 Doberman Pinschers with preclinical dilated cardiomyopathy (the PROTECT
597 Study). *Journal of veterinary internal medicine* **26**, 1337–1349
598 Thomas, R.E. (1987) Congestive cardiac failure in young Cocker Spaniels (a form of

599 cardiomyopathy?): details of eight cases. *Journal of Small Animal Practice,*
600 28(4), pp.265-279
601 Thomas, W.P., Gaber, C.E., Jacobs, G.J., *et al.* (1994) Recommendations for
602 standards in transthoracic two-dimensional echocardiography in the dog and cat.
603 *Veterinary radiology & ultrasound: the official journal of the American College of*
604 *Veterinary Radiology and the International Veterinary Radiology Association* **35**,
605 173–178
606 Tidholm, A., Svensson, H. & Sylvén, C. (1997) Survival and prognostic factors in 189
607 dogs with dilated cardiomyopathy. *Journal of the American Animal Hospital*
608 *Association* **33**, 364–368
609 Tôrres, C. L., Backus, R. C., Fascetti, *et al.* (2003). Taurine status in normal dogs fed
610 a commercial diet associated with taurine deficiency and dilated
611 cardiomyopathy. *Journal of animal physiology and animal nutrition,* 87(9-10),
612 359-372.
613 Van Vleet, J.F. and Ferrans, V.J., (1986) Myocardial diseases of animals. *The*
614 *American journal of pathology,* 124(1), p.98.
615 Vollmar, A.C., Fox, P.R., Servet, E., *et al.* (2013) Determination of the prevalence of
616 whole blood taurine in Irish wolfhound dogs with and without echocardiographic
617 evidence of dilated cardiomyopathy. *Journal of veterinary cardiology: the official*
618 *journal of the European Society of Veterinary Cardiology* **15**, 189–196
619

Figures

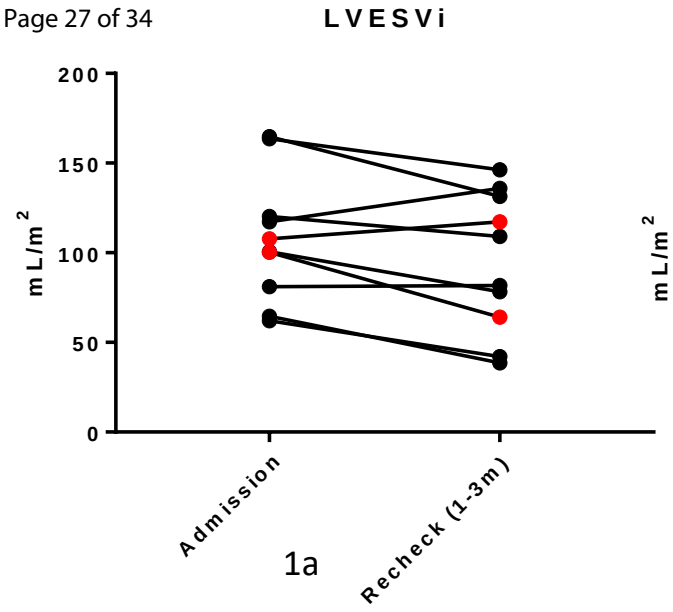
Fig. 1 Line chart of the echocardiographic values at admission and at the first recheck. Only dogs with available echocardiographic values are shown. The dogs with normal taurine levels are indicated with red dots. The horizontal line is the mean value.
1a) LVESVi: left ventricular end-systolic volume indexed to body surface area. 1b) LVEDVi: left ventricular end-diastolic volume indexes to body surface area. 1c) LVIDDN: left ventricular internal dimension in diastole normalized for body weight. 1d) LVIDSN: left ventricular internal diameter in systole normalized for body weight. 1e) EF: ejection fraction. 1f) FS: fractional shortening. 1g) LVIDd: left ventricular internal dimension in diastole. 1h) EPSS: Mitral M-mode E-point septal separation.

Fig 2a Echocardiographic 2D image of one of the dogs with low taurine concentration before starting supplementation. Right parasternal long-axis 4-chambers view showing severe left ventricle dilation. EF: 36%. **Fig 2b** Echocardiographic M-Mode image of one of the dogs with low taurine concentration before starting supplementation. Right parasternal short-axis view at the level of the papillary muscles showing poor systolic function and dilated left ventricle. FS: 11%; LVESVi: 102.9 mL/m^2; LVIDSN: 2.07; LVIDDN: 2.47. **Fig 2c** Echocardiographic 2D image of one of the dogs with low taurine concentration after starting supplementation (3 months). Right parasternal long-axis 4-chambers view showing less severe left ventricle dilation. EF: 47%. **Fig 2d** Echocardiographic M-Mode image of one of the dogs with low taurine concentration after starting supplementation (3 months). Right parasternal short-axis view at the level of the papillary muscles showing improved systolic function and left ventricular dimensions. FS: 7%; LVESVi: 64.2 mL/m^2; LVIDSN: 1.07; LVIDDN: 1.93.
Abbreviations: EF: ejection fraction. FS: fractional shortening. LVESVi: left ventricular end-systolic volume indexed to body surface area. LVIDSN: left ventricular internal diameter in systole normalized for body weight. LVIDDN: left ventricular internal diameter in diastole normalized for body weight.

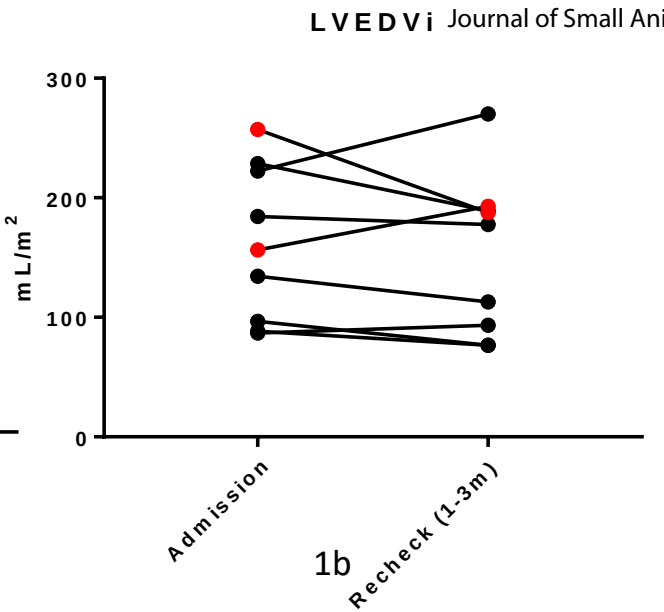
Fig. 3a Graphic representation of the left ventricular end-systolic volume indexed to body surface area of all dogs with echocardiographic follow-up values. **Fig. 3b** Graphic representation of the left ventricular internal dimension in systole normalized for body weight of all dogs with echocardiographic follow-up values.
Red dots indicate dogs with normal taurine levels. Each dot indicates an echocardiographic examination.
Abbreviations: LVESVi: left ventricular end-systolic volume indexed to body surface area. LVIDSN: left ventricular internal diameter in systole normalized for body weight.

Fig. 4 Kaplan Meier survival curve of the entire population of dogs included in the study.

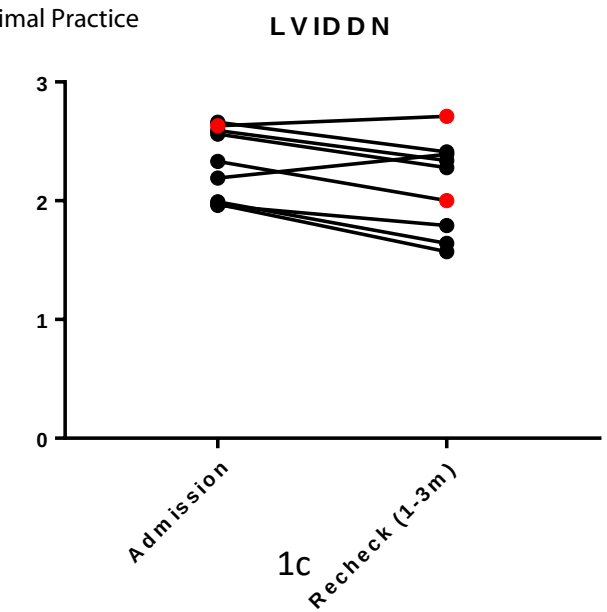
663
664
665
666
667



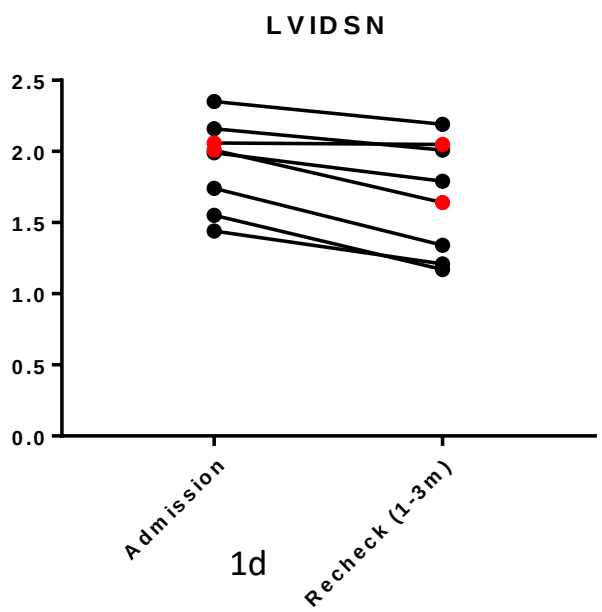
1a



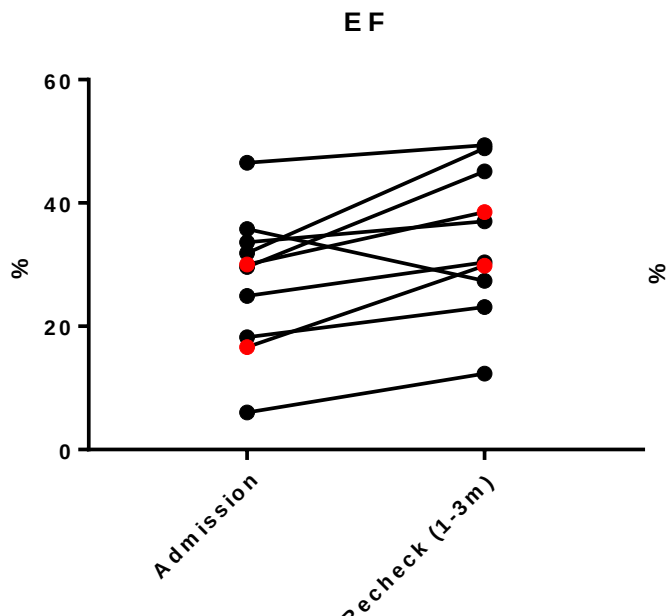
1b



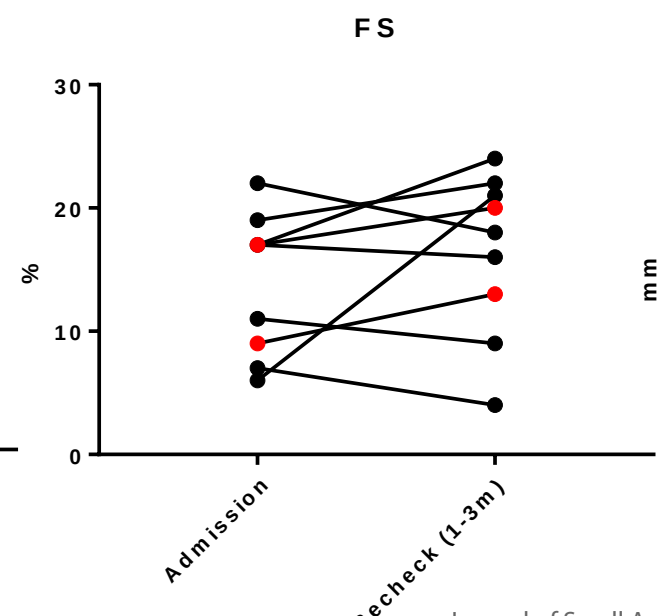
1c



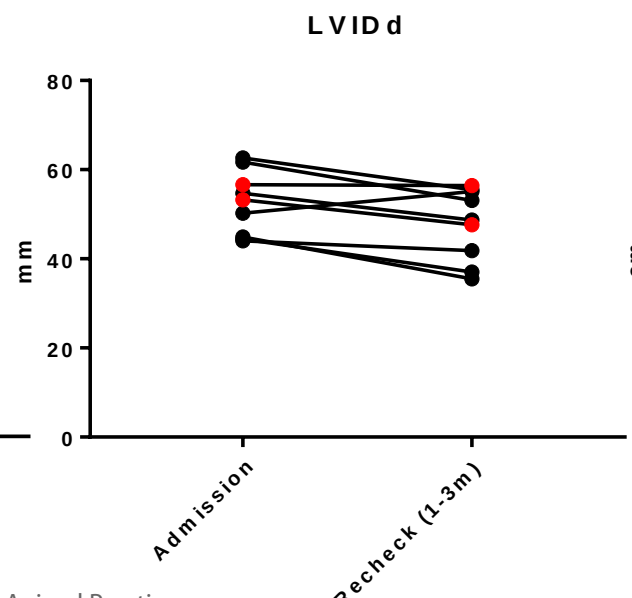
1d



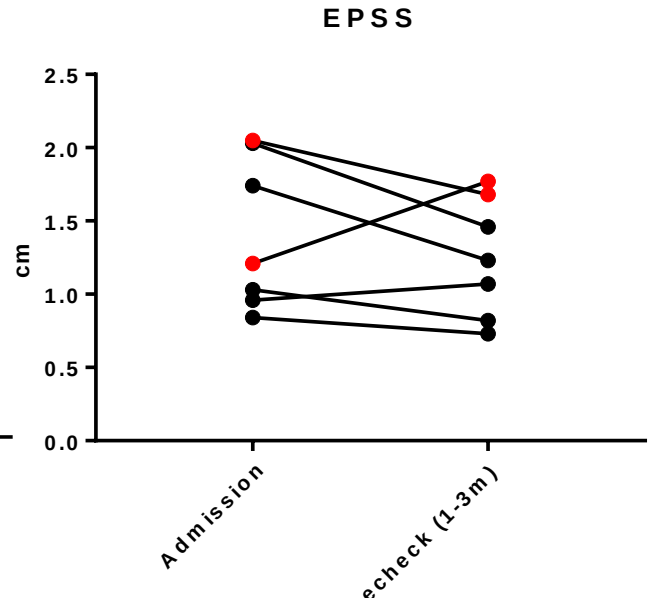
1e



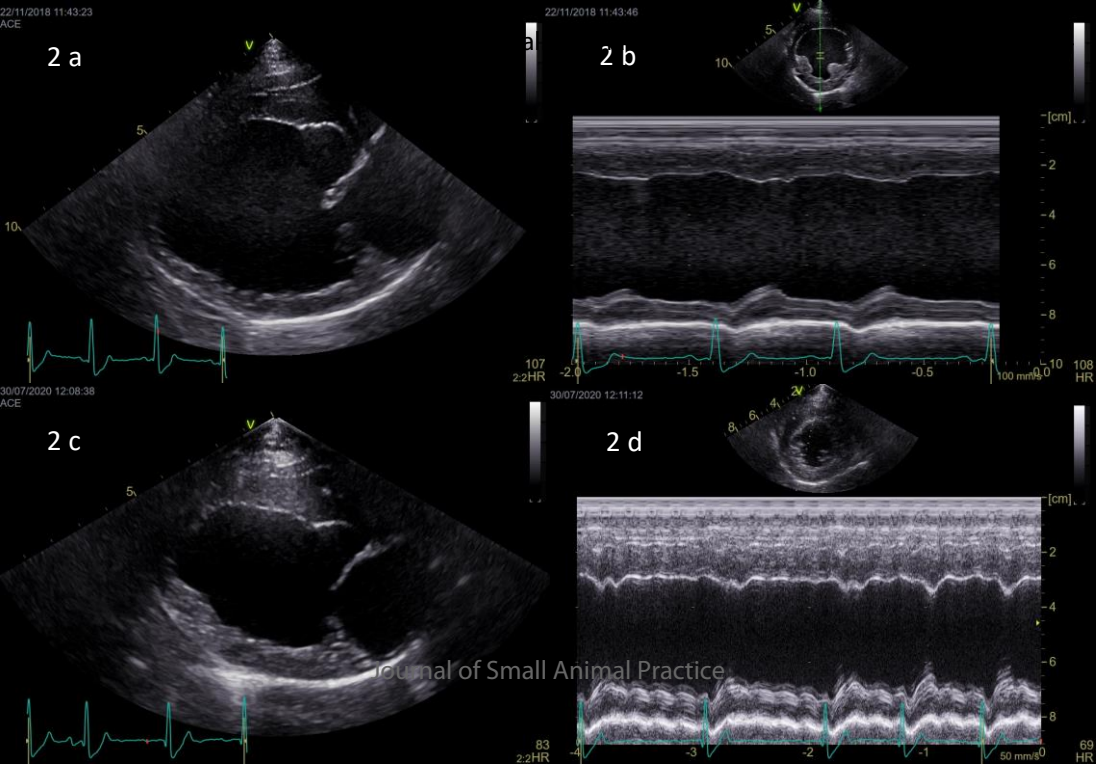
1f

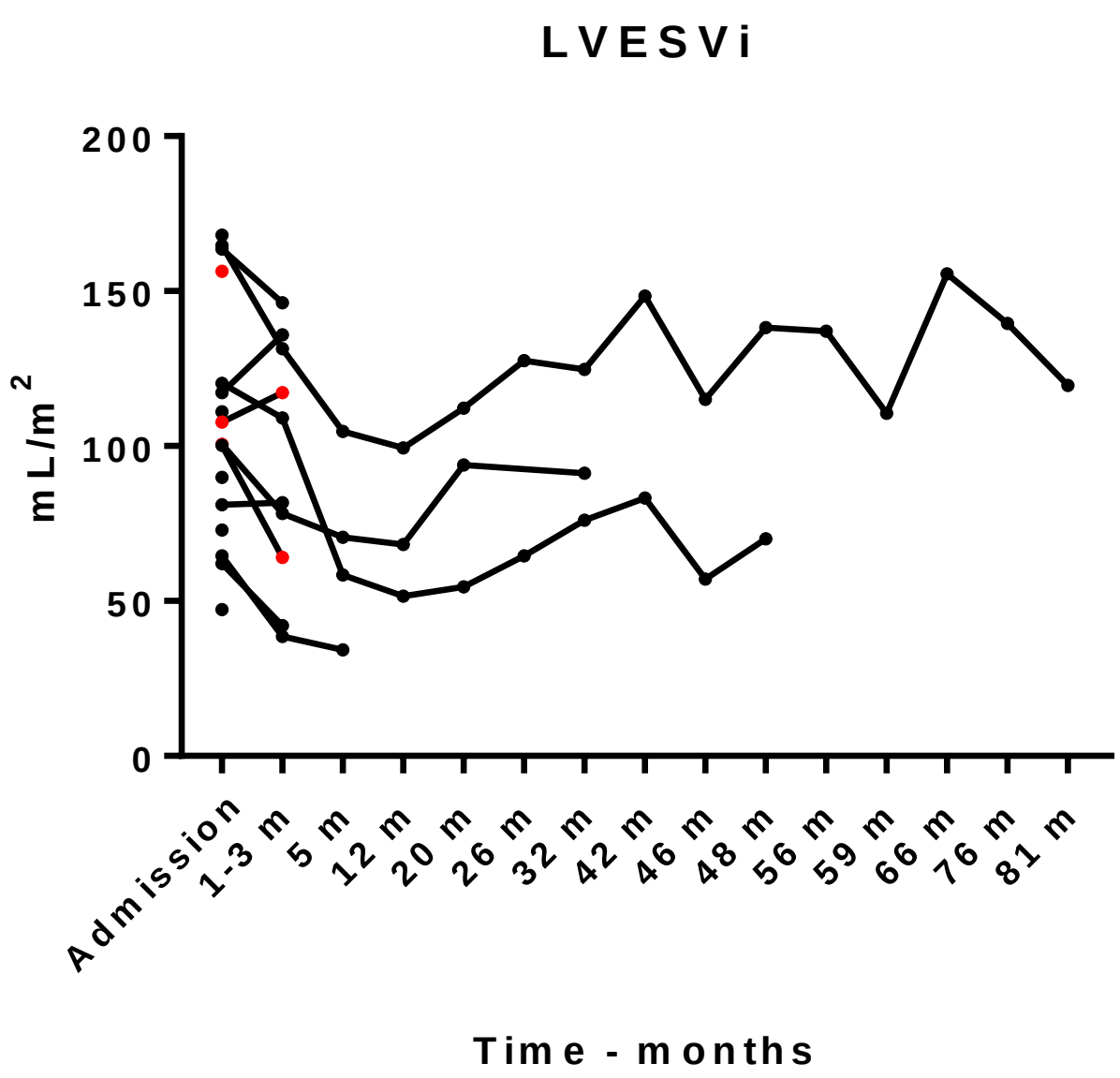


1g

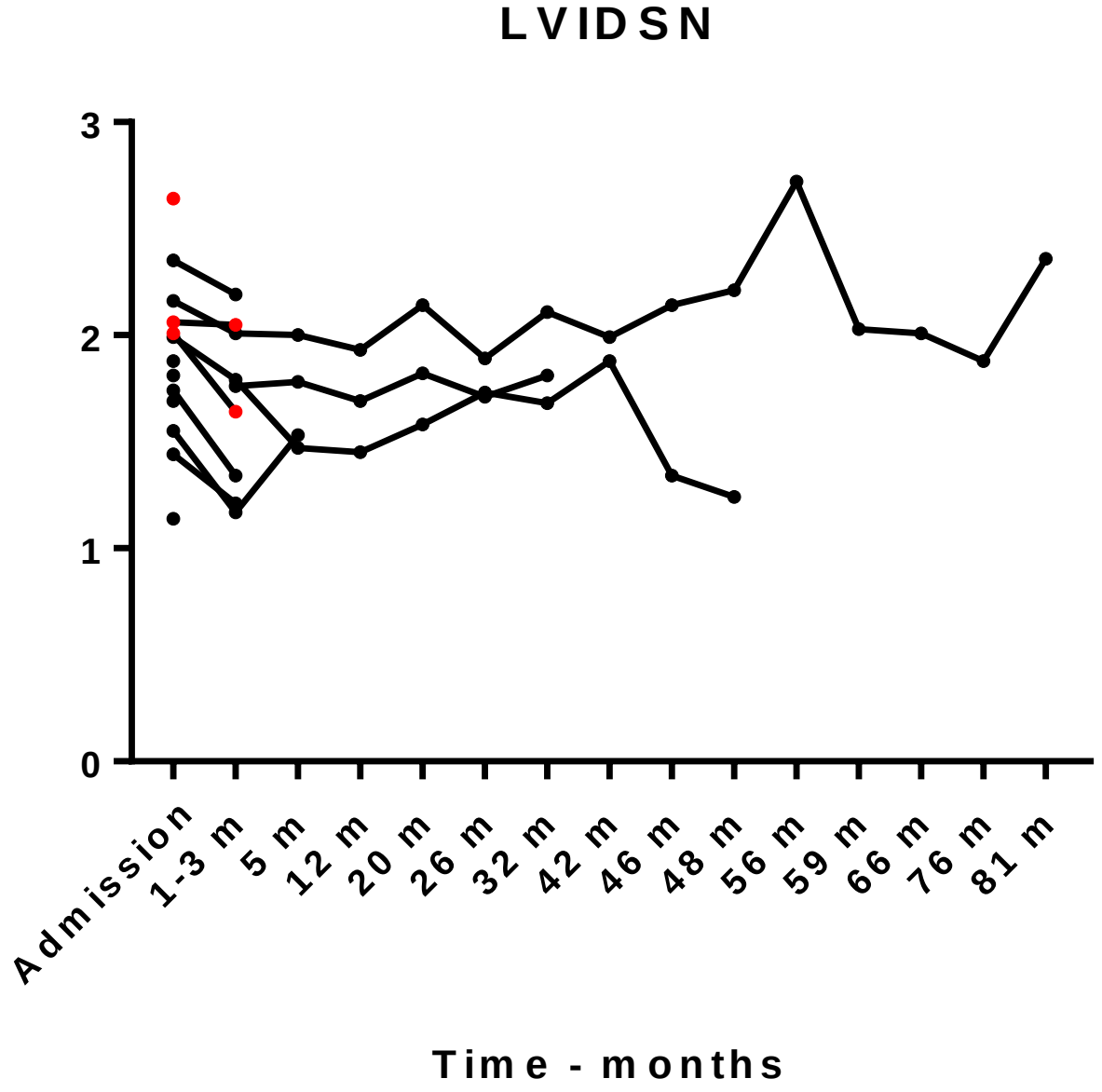


1h




LVESVi
200
150
100
50
0
mL/m2
Admission
1-3 m
5 m
12 m
20 m
26 m
32 m
42 m
46 m
48 m
56 m
59 m
66 m
76 m
81 m
Time - months

3a


LVIDSN
3
2
1
0
Admission
1-3 m
5 m
12 m
20 m
26 m
32 m
42 m
46 m
48 m
56 m
59 m
66 m
76 m
81 m
Time - months

3b

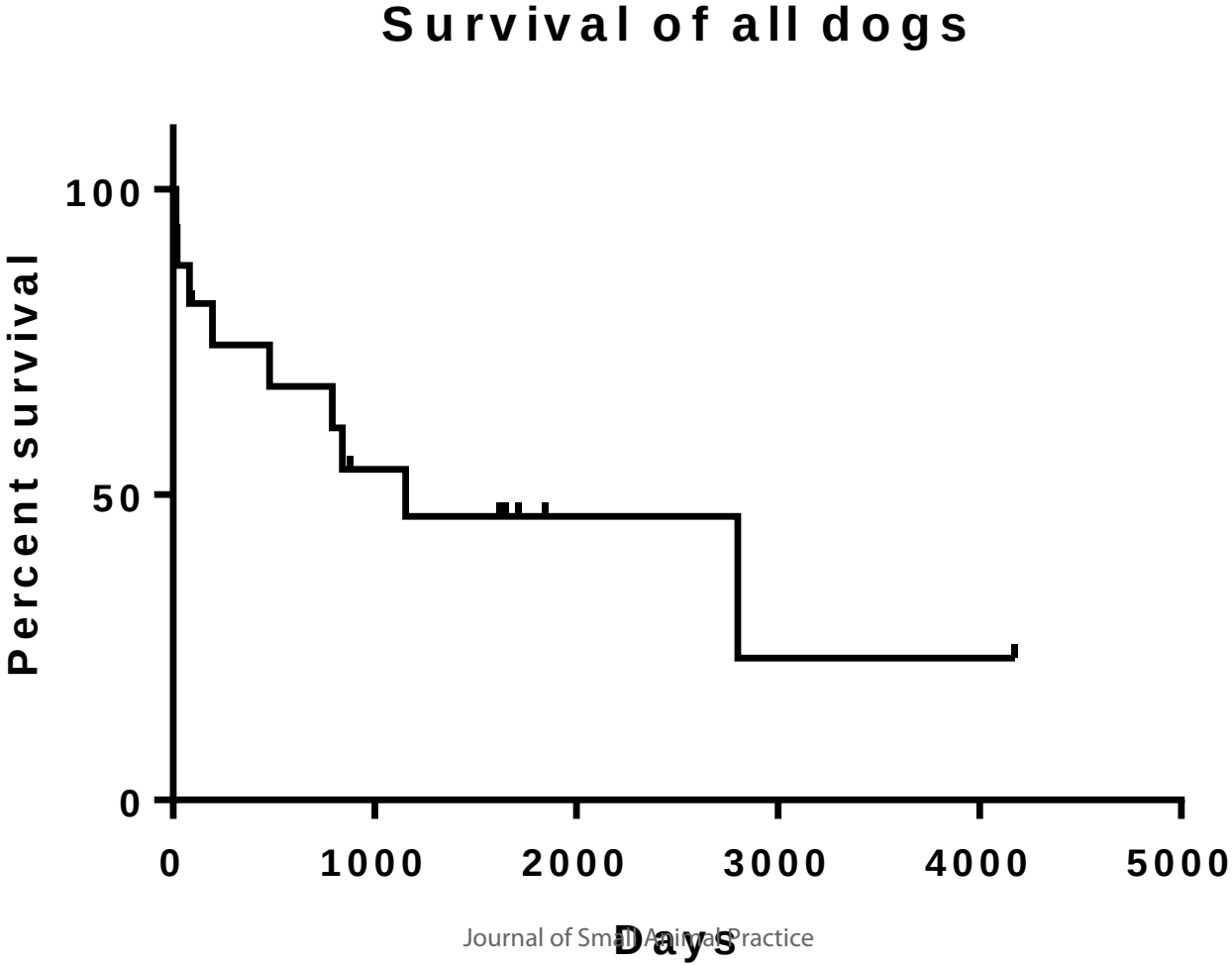

Survival of all dogs
Percent survival
100
50
0
0
1000
2000
3000
4000
5000
Days
Journal of Small Animal Practice

Table 1. Signalment, taurine concentrations, medications received, outcome and survival time of all dogs included in the study.

	Group	Age	Sex	Weight	Taurine plasma concentration (ref. 50-180 umol/L)	Taurine plasma concentration 6 months after supplementation	Pimobendan (mg/kg/day)	Furosemide (mg/kg/day)	Spironolactone (mg/kg/day)	Benazepril (mg/kg/day)	Taurine supplementation (mg/kg/day)	Additional medications	First recheck interval (days)	Outcome	Survival time (days)
Dog 1	LTC	1	MN	17.5	22					0.6	66		NA	Lost to follow-up	NA
Dog 2	LTC, CHF	9	MN	16.7	2		0.6	7.5	1	0.3	30		NA	Lost to follow-up	NA
Dog 3	LTC, CHF	7	FN	17	6	200	1	7.5	2.5	0.33	116		90	Died of cardiac death	790
Dog 4	LTC, CHF	4	M	16	26		0.6	1.2		0.33	66	Carnitine	NA	Died of cardiac death	1155
Dog 5	LTC, CHF	8	FN	15.4	39		0.6	3.6		0.3	122	Diltiazem Levothyroxine	90	Alive	1550
Dog 6	LTC, CHF	9	MN	14.7	9		0.6	9.9	2.5	0.3	17	Hydrochlorothiazide Amiloride	7	Lost to follow-up	NA
Dog 7	LTC, CHF	3	FE	9.5	29		1	6	1	0.5	50		NA	Died of cardiac death	20
Dog 8	LTC, CHF	7	FN	15	9		0.6	6.6	2.2	0.33	33	Amlodipine	30	Lost to follow-up	NA
Dog 9	LTC, CHF	4	MN	20.8	5		0.5	6	2	0.25	50		30	Alive	2430

Dog 10	LTC	10	M	15	27		0.6				66		30	Died of cardiac death	840
Dog 11	LTC, CHF	8	FN	11.7	19		0.5	9	4	0.5	150	Clopidogrel	60	Died of cardiac death	195
Dog 12	LTC	9	ME	14.7	19		0.3				66	Diltiazem Clopidogrel	NA	Died of cardiac death	83
Dog 13	LTC, CHF	2	ME	18.9	15	279	0.4	6	2.5	0.3	50		90	Died of cardiac death	2800
Dog 14	NTC, CHF	11	MN	13.5	75		0.8	9	1.2	0.4	80	Hydrochlorthiazide Amiloride	30	Died of cardiac death	478
Dog 15	NTC, CHF	9	MN	13.6	194		0.5	6	1.3	0.33		Clopidogrel Sildenafil Kaminox Doxycycline	90	Died of cardiac death	14
Dog 16	NTC, CHF	7	ME	16.5	81		0.5	5	2.3	0.3			90	Alive	910

Dog n° 14 had taurine concentrations close to the lower reference interval and was supplemented. Dog n° 4 received carnitine despite lack of concentration measurements. Abbreviations: ME: male entire. MN: male neutered. FE: female entire. FN: female neutered. LTC: low taurine concentration. NTC: normal-taurine concentration. CHF: congestive heart failure.

Table 2. Mean echocardiographic values of the dogs included in the study at admission and at the first recheck after admission; subdivided in all population, LTC and NTC.

ALL POPULATION	Variables at admission: mean (± standard deviation; SD)	First recheck after admission: mean (± SD)
LVIDd (mm)	51.55 (± 9.6)	49.92 (± 10.6)
LVIDDN	2.32 (± 0.45)	2.26 (± 0.53)
LVIDSN	1.88 (± 0.39)	1.68 (± 0.37)
LVESVi (mLs/m^2)	107.9 (± 38.8)	94.4 (± 39.0)
LVEDVi (mLs/m^2)	153.9 (± 60.78)	152.9 (± 66.38)
Ejection fraction (%)	27.4 (± 10.6)	34.1 (± 11.89)
Fractional shortening (%)	14.89 (± 5.94)	16.62 (± 7.43)
EPSS (mm)	15.5 (± 5.1)	14.5 (± 5.0)
LTC		
LVIDd (mm)	49.69 (± 8.7)	46.67 (± 6.5)
LVIDDN	2.22 (± 0.38)	2.06 (± 0.37)
LVIDSN	1.77 (± 0.35)	1.63 (± 0.40)
LVESVi (mLs/m^2)	104.8 (± 40.8)	95.4 (± 41.8)
LVEDVi (mLs/m^2)	148 (± 63.1)	140 (± 69.1)
Ejection fraction (%)	28.1 (± 11.4)	34.1 (± 13.2)
Fractional shortening (%)	14.87 % (± 6.37)	16.28 (± 7.31)
EPSS (mm)	14.4 (± 5.0)	11.8 (± 4.5)

NTC (individual values)		
LVIDd (mm)	56.6 / 68.8 / 53.2	56.4 / 47.6
LVIDDN	2.63 / 3.19 / 2.33	2.71 / 2
LVIDSN	2.06 / 2.64 / 2.01	2.05 / 1.64
LVESVi (mLs/m^2)	107.8 / 156.4 / 100.2	117.3 / 64.1
LVEDVi (mLs/m^2)	156.4 / 215.7	192.9
Ejection fraction (%)	30 / 26.8 / 16.6	38.5 / 29.8
Fractional shortening (%)	17 / 13 / 9	20 / 13
EPSS (mm)	2.05 / 2.07 / 1.21	1.68 / 1.77

At the recheck 5 dogs in the LTC did not have values available.

Abbreviations: LTC: low taurine concentration. NTC: normal-taurine concentration. LVIDd: left ventricular internal diameter in diastole. LVIDDN: left ventricular internal diameter in diastole normalized for body weight. LVIDSN: left ventricular internal diameter in systole normalized for body weight. LVESVi: left ventricular end-systolic volume indexed to body surface area. LVEDVi: left ventricular end-diastolic volume indexed to body surface area. EPSS: Mitral M-mode E-point septal separation. NA not applicable.